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LONG-TERM FOLLOW UP OF T CELL DEPLETED TRANSPLANTS FROM UNRELATED DONORS IN PEDIATRIC PATIENTS

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Graft-versus-host disease (GVHD) remains a major cause of mortality and long term morbidity in recipients of hematopoietic stem cell transplant from unrelated donors. From 1993-2000 we used partial T cell depletion to reduce this risk and transplanted 116 patients with T cell depleted unrelated donor stem cells on two successive IRB-approved protocols at St Jude Children's Research Hospital (1993-1997 n = 75) and Baylor College of Medicine (1998-2000 n= 41). All patients were transplanted for hematologic malignancy and were stratified into standard risk (Acute leukemia or lymphoma in 1st or 2nd remission or CML in 1st chronic phase) or high risk (Acute leukemia or lymphoma in relapse or ≥ CR3, CML beyond 1st chronic phase, myelodysplasia or secondary AML). Patients received marrow from 5/6 or 6/6 matched unrelated donors depleted of T cells by incubation with CD6 and CD8 antibodies and baby rabbit complement, which produced a median T cell depletion of 96%. Conditioning was with Cyclophosphamide 45mg/kg x 2, Ara-C 3g/m^2 x 6, ATG 30mg/kg x 3 and TBI 1200-1400cGy. In 1997 we reported initial outcome data in the first 51 patients (Hongeng et al Lancet 1997, 350: 767-71) with a 2-year disease-free survival estimate for standard-risk recipients of 73±12.1% and for high risk recipients of 32 ± 15.1%. We now report long term follow up on all 116 patients with follow up ranging between 6.7 and 13.5 years. The 5 and 10 year Kaplan Meier survival estimate is 60% for 49 standard risk patients and 37% for 67 high risk patients. 4 of the 49 standard risk patients and 26 of the 67 high risk patients relapsed. Three of the patients who relapsed are long term survivors after relapse (> 10 years) after donor lymphocyte infusion. All but one relapse occurred within 2 years of transplant. The incidence of grade 3-4 GVHD was 5% and only 4% of recipients developed extensive chronic GVHD. The 100 day mortality was 21% in high risk recipients and 19% in standard risk recipients but the incidence of late non-relapse mortality was low with only two deaths from causes other than relapse after one year - one death from pulmonary failure at 4.5 years and one at 10 years in a motor vehicle accident. All long term survivors have a good performance status. Partial T cell depletion can therefore reduce the risk of graft versus host disease and long term sequelae from this complication without an increased risk of relapse.

IMMUNE RECONSTITUTION

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INSULIN-LIKE GROWTH FACTOR I POSITIVELY REGULATES THYMIC FUNCTION BY EXPANSION OF THYMOCYTE PRECURSORS AND THYMIC EPITHELIAL CELLS

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Neuroendocrine growth factors help regulate thymic function and serve as potential agents to accelerate thymic T-cell production following hematopoietic stem cell transplantation (HSCT). We present evidence supporting insulin-like growth factor I (IGF-1) as a thymic regulator. Continuous infusion of IGF-1 (100 ug/day) into normal mice resulted in significant increases in all thymocyte populations, including earliest thymocyte precursors (ETP), with subsequent increases in recent thymic emigrants (RTE). Time-sequential enumeration of thymic epithelial cell (TEC) and thymocyte subpopulations and bone marrow and peripheral LSK (Lineage-, Sca-1+, c-kit+) precursor populations showed that expansion of peripheral LSK, occurring on day 4 of IGF-1 administration, preceded quantitative increases in thymocyte and TEC subpopulations by three days. Concomitant with the increase in peripheral LSK numbers, cell cycle entry was increased in bone marrow and peripheral LSK and lineage-CD44+ CD25+ (DN2) thymocytes. IGF-1 administration also affected TEC turnover during this early time period (day 2-7) preceding numeric increases in TEC. The relative proportions of cortical and medullary TEC were not altered throughout the course of IGF-1 administration. Finally, mice lacking IGF-1 receptor (IGF-1R) signaling on T-cells were generated through cre-mediated deletion of the IGF-1R high-affinity binding site (pLCK-cre/loxIGF1R). Compared to wild-type littermates, pLCK-cre-loxIGF1R mice exhibited a decrease in the number of CD4$^+$CD8$^+$ thymocytes, thymic TREC, and splenic naïve T-cells and RTE. IGF-1 treatment, however, restored thymocyte and peripheral subset numbers in these mice. These results demonstrate: 1) IGF-1 expands thymocyte subpopulations and increases thymic output; 2) IGF-1 expands peripheral thymocyte precursor populations leading to their increased availability for entry into the thymus; and 3) IGF-1R signaling is required for the maintenance of normal thymocyte and peripheral T-cell populations, and that presumptive IGF-1 effects on TEC can overcome the absence of IGF-1R signaling in thymocytes. Together, the results support the concept of neuroendocrine growth factors such as IGF-1 in preserving and/or enhancing thymic function recovery following HSCT, and suggest that points of regulation in thymic function by IGF-1 include entry of thymocyte precursors into the thymus and the proportionate expansion of TEC populations that facilitate thymocyte development.

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A SUBPOPULATION OF HUMAN NK CELLS LACKING INHIBITORY RECEPTORS FOR SELF MHC IS DEVELOPMENTALLY IMMATURE RATHER THAN AUTOREACTIVE

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The effector function of human natural killer (NK) cells is down-regulated via ligation of inhibitory receptors (killer immunoglobulin-like receptors [KIR] and NKG2A) that recognize self MHC. In order to study the mechanism producing self-tolerance, which is not yet understood., we developed and validated a quantitative, real-time RT-PCR (Q-RT-PCR) assay to measure mRNA levels from individual *NKG2* and *KIR* genes. Our expression typing assay accurately predicts genotyping by SSOP (sensitivity 0.94. specificity 0.96, PPV 0.97) and gives expression data with a single quantitative readout. We used this assay to investigate NK cells circulating in normal blood. We sorted CD56^{+dim} NK cells into KIR$^+$ and KIR$^-$ subsets by flow cytometry using an antibody cocktail recognizing 6 KIR. Measurements of individual *KIR* gene expression showed that not all CD56^{+dim} cells express KIR. The KIR$^-$ populations were further divided into NKG2A positive and negative cells, defining a novel subpopulation of cells committed to the NK lineage. This KIR$^-$ NKG2A$^-$ subset comprises 19.4 ± 2.8% of CD56^{+dim} NK cells in healthy donors, and expresses the activating NKG2D and NKG2E receptors. Consequently these CD56^{+dim} NKG2A$^-$KIR$^-$ NK cells do not have 'at least one' inhibitory receptor for engaging autologous MHC class I. However, they are not intolerant, autoreactive cells, but instead are immature, already committed NK cells (based on CD56 expression), coexpressing CD7, CD16, and CD18. Functional assays showed this population to be hyporesponsive. Compared to KIR or NKG2A-expressing subsets, they exhibited impaired degranulation (measured by CD107a) and poor cytotoxicity against K562 targets. Furthermore, they produced little IFN-γ after stimulation with IL-12 and IL-18, and compared to that of CD56^{+dim} NK cells that express NKG2A, they showed a diminished capacity to proliferate in response to IL-15. Upon culture on a murine embryonic stromal cell line with IL-15, these CD56^{+dim} NKG2A$^-$KIR$^-$ NK cells

proliferate, express KIR and NKG2A receptors and develop cytotoxic and cytokine-producing potential. Umbilical cord blood, a site of developing hematopoiesis, contained a significantly higher percentage of $CD56^{+dim}$ $NKG2A^{-}KIR^{-}$ cells (59 ± 11%) supporting the notion that these cells are developmentally immature. We conclude that lineage-committed NK cells in the blood do not require inhibitory self-tolerance mechanisms until they reach a late stage of differentiation.

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PRE-CLINICAL STUDY OF THE EFFECT OF THE AS-SIG-TAA/ECDCD40L VECTOR PRIME-TAA/ECDCD40L PROTEIN BOOST VACCINE IN ELDERLY RECIPIENTS FOR SUPPRESSION OF RECURRENT CANCER FOLLOWING ALLOGRAFTING AND DONOR LYMPHOCYTE INFUSIONS (DLI)

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The mini-allogeneic transplant has opened the door to treating individuals in the fifth and sixth decades of life. Unfortunately, matched related donors for these individuals usually have an aged immune system in which the immune response has been impaired by a reduction in the ratio of antigen naïve/memory CD4 and CD8 T cells and acquired functional defects in activated "helper" CD4 T cells (eg diminished CD40 ligand (CD40L) expression). This has limited the applicability of mini-allografts to older individuals and to the use of post allograft vaccines to expand specific populations of CD8 effector cells to bolster the anti-cancer and anti-viral immune response. Our laboratory has developed an adenoviral vector (Ad-sig-TAA/ecdCD40L) vaccine which is designed for the in vivo target associated antigen (TAA) loading and activation of dendritic cells (DCs), and to overcome the absence of CD40L expression in activated CD4 helper T cells in older individuals. The subcutaneous (sc) injection of this vector leads to the release of a fusion protein composed of a TAA linked to the extracellular domain (ecd) of the CD40 ligand (CD40L), which binds to the CD40 receptor on DCs, activates the DCs, and leads to the presentation of TAA fragments on Class I MHC. VPP vaccine overcomes anergy in TAA.Tg transgenic mouse, and induces TAA specific memory cells. Two sc injections of the TAA/ecdCD40L protein as a booster following the sc administration of the Ad-sig-TAA/ecdCD40L vector (VPP) expands the magnitude of the cellular and humoral immune response induced by the vector in 18 month old aged mice as well as in younger mice. This vaccine decreased levels of negative regulatory CD4 FOXP3 T cells in tumor nodules. We administered TBI and an allogeneic stem cell transplant 7 days post sc injection of the E7 positive TC-1 cells. DLI from an Ad-sig-E7/ecdCD40L vector prime-E7/ecdCD40L protein boost vaccinated donor were injected iv 3 days post transplant, and a single E7/ecdCD40L protein boost sc vaccination one week thereafter. We found that the growth rate of the E7 positive TC-1 tumor cells post allograft was less in the vaccinated than in the control (injection of tumor cells followed in 7 days by TBI), or the animals in which the allograft recipient was vaccinated without DLI. Thus, the use of DLI from VPP vaccinated allodonor decreased tumor cell growth post allograft.

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DIRECT ISOLATION AND INFUSION OF DONOR-DERIVED CMV-SPECIFIC T CELLS FOR TREATMENT OR PROPHYLAXIS OF CMV INFECTION FOLLOWING ALLOGENEIC TRANSPLANTATION

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Reactivation of CMV is common following allogeneic HSCT and virus-specific T lymphocytes are necessary for control. CMV-specific donor T cell infusions have been used but most methods involve several weeks of ex-vivo culture. The current method involves a 20hr incubation of donor peripheral blood mononuclear cells with rCMV-pp65 protein. Isolation of interferon-gamma positive T cells by CliniMACS using IFNγ capture microbeads (Miltenyi Biotec) provides a CMV-reactive T cell product which is cryopreserved in dosed aliquots for subsequent infusion. A single arm phase I study is underway, with CMV-T cells given pre-emptively at first detection (by qPCR) of CMV DNA in peripheral blood, or at day +40-50 as prophylaxis. A dose of 1×10^4 $CD3^+$/kg recipient weight is infused and CMV monitored by weekly PCR. Antiviral drug therapy commences if the viral load rises above our institutional threshold. 12 patients have received CMV-T cells at a median of 4 weeks post HSCT and 9 are alive and well. None experienced infusion-related toxicity and no deaths were associated with CMV-T cell treatment or CMV disease. Incidence and severity of GvHD was no different from historical controls. The median yield of CMV-T cells following enrichment was 5.2×10^6 (range 0.29-26.7) of which 24.7% were $CD4^+/IFN\gamma^+$ and 11.2% were $CD8^+/IFN\gamma^+$; a total mean CMV-reactive cell yield of 2.2 $\times10^6$ per donor. Following infusion, in vivo expansion of CMV-T cells was seen in all patients. CMV-T cells averaged 9.8% of $CD4^+$ cells and 8.1% of $CD8^+$ cells by 2-4 weeks post-infusion; the result of in vivo expansions of CMV-reactive cells of up to 5000-fold. 9 patients received antiviral therapy for CMV reactivation but in 5 patients this was required for a significantly shorter period than in historical controls (11-14 days). 3 patients had a second CMV reactivation. 1 patient showed substantially delayed expansion of CMV-T and required prolonged anti-viral treatment (33 days). 1 required antiviral drug treatment, the second received no treatment and cleared virus after a further in vivo expansion of CMV-T cells, suggesting the presence of a functional memory population. 3 patients were treated prophylactically at day 40-50 and expansions of CMV-reactive T cells were seen in all 3 despite lack of detectable viral DNA in peripheral blood. This technique rapidly provides clinical-grade CMV-reactive $CD4^+$ and $CD8^+$ T cells which appear to provide effective antiviral immunity.

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THYMIC SHIELDING (TS) IN RECIPIENTS OF TOTAL BODY IRRADIATION (TBI) AND ALTERNATIVE DONOR HEMATOPOIETIC STEM CELL TRANSPLANT (AD-HSCT): REDUCED RISK OF OPPORTUNISTIC INFECTION IN PATIENTS WITH FANCONI ANEMIA (FA)

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Delayed immune reconstitution and consequent opportunistic infections remain major obstacles to the successful application of HSCT, particularly in older patients, those with HLA mismatched donors and in selected diseases, such as FA. Based on preclinical work suggesting that TS may improve immune reconstitution in recipients of TBI and allogeneic HSCT (J Immunol 1987:139:358), we evaluated the safety and potential efficacy of TS in FA patients. After CT localization of the thymus, 5HVL cerrubend blocks were fabricated and used to shield the thymus. Otherwise all patients received the standard preparative regimen consisting of fludarabine 175 mg/m2, cyclosphosphamide 40 mg/kg, single fraction TBI 450 cGy, and antithymocyte globulin, with cyclosporine and short course methylprednisolone as GVHD immunoprophylaxis. In order to assess the potential risks and benefits of TS, we compared transplant outcomes of these FA patients who received TS to FA patients treated with the exact same preparative regimen without TS. Between April 1999-June 2006, 59 FA patients underwent AD-HSCT at the University of Minnesota; 16 patients had TBI with TS and 43 had TBI without TS. For those with and without TS, donors were HLA matched (n=42) or mismatched (n=17), and stem cell sources were T cell depleted bone marrow (n=9 vs n=38) or umbilical cord blood (n=7 vs n=5). While excess graft failure was considered to be the principal toxicity risk in recipients of TS, incidence of engraftment was similar in those with and without TS (94% vs 97% respectively, p=.46). Although not statistically significant, survival at one year was higher in FA patients with TS (67% vs 53% respectively, p=.46). However, as shown, TS was associated with a significantly lower risk of all three categories of opportunistic infection after HSCT (Table 1).